

# Temperature effect on the small-to-large crossover lengthscale of hydrophobic hydration

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## Abstract.

The thermodynamics of hydration is expected to change gradually from entropic for small solutes to enthalpic for large ones. The small-to-large crossover lengthscale of hydrophobic hydration depends on the thermodynamic conditions of the solvent such as temperature, pressure, presence of additives, etc... We attempt to shed some light on the temperature dependence of the crossover lengthscale by using a probabilistic approach to water hydrogen bonding that allows one to obtain an analytic expression for the number of bonds per water molecule as a function of both its distance to a solute and solute radius. Incorporating that approach into the density functional theory, one can examine the solute size effects on its hydration over the entire small-to-large lengthscale range at a series of different temperatures. Knowing the dependence of the hydration free energy on the temperature and solute size, one can also obtain its enthalpic and entropic contributions as functions of both temperature and solute size. These function can provide some interesting insight into the temperature dependence of the crossover lengthscale of hydrophobic hydration. The model was applied to the hydration of spherical particles of various radii in water in the temperature range from  $T = 293.15$  K to  $T = 333.15$  K. The model predictions for the temperature dependence of the hydration free energy of small hydrophobes are consistent with the experimental and simulational data on the hydration of simple molecular solutes. The  $T$ -dependence of the crossover radius is approximately linear if a linear approximation is used for the  $T$ -dependence of the hydration free energy resulting from numerical results.

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# 1 Introduction

Hydrophobic effects, i.e., hydrophobic hydration and hydrophobic interactions, are believed to constitute an important (if not crucial) element of a wide variety of physical, chemical, and biological phenomena,<sup>1–10</sup> such as the immiscibility of oil and water, micelle and membrane formation, the formation, stability, and unfolding of the native structure of a biologically active protein, etc... Hydrophobic hydration is the thermodynamically unfavorable dissolution of a hydrophobic particle (microscopic or macroscopic), whereof the accommodation in water is accompanied by an increase in the associated free energy due to structural (and possibly energetic) changes in water around the hydrophobe. Since the total volume of water affected by two hydrophobes is smaller when they are close together than when they far away from each other, there appears an effective, solvent-mediated attraction between them which is referred to as hydrophobic attraction. Most properties of hydrophobic interactions may be unambiguously determined from the analogous properties of hydrophobic hydration; the former can be regarded as a partial reversal of the latter.

Both hydrophobic hydration and hydrophobic interactions have been the subject of intensive theoretical, simulational, and experimental research for several decades.<sup>11–14</sup> Still the understanding of many aspects of these effects remains rather unsatisfactory (sometimes even contradictory visions of the same issue arise from different research reports). For example, although the dependence of hydrophobic effects on both the solvent temperature and the hydrophobe size is not contested, many thermodynamic and molecular details thereof are still to be elucidated.<sup>5–8,15–17</sup>

The temperature and length-scale dependence of hydrophobic effects clearly transpires in two exciting problems of modern biophysics, namely, protein folding and protein denaturation.<sup>5–8,15–18</sup> Upon folding, a protein buries its nonpolar amino acids into a globular core, away from contact with water, to form a “native” configuration in which the protein is biologically functional; the burying of

amino acids occurs in a particular temporal and spatial order depending, among other factors, on their sizes. The strength of hydrophobic interactions largely determines the temperature range where the native configuration remains stable (hence protein physiologically active) before its thermal unfolding (denaturation) occurs. The (amino acid) size-dependent weakening of hydrophobic interactions at lower temperatures was suggested<sup>8,18,19</sup> to be an important factor in the cold denaturation of proteins.

In simplified terms, the hydrophobicity of a solute particle can be regarded as a consequence of an unfavorable entropy change that either overweighs a favorable energy change or supplements the unfavorable energy change, both occurring upon the accommodation of the particle in water. That is, the entropic contribution to the free energy change upon hydration is always positive while the enthalpic contribution thereto can be either negative (for small enough solutes) or positive (for larger ones). The actual mechanism of hydrophobic effects depends on the size and nature of solute particles involved as well as on thermodynamic conditions imposed on the solvent (temperature, pressure, etc... ).

The hydration of small hydrophobic molecules (of sizes comparable to a water molecule) is believed to be entropically “driven” (and so is their solvent-mediated interaction) at all temperatures.<sup>13,14</sup> Such molecules can fit into the water hydrogen-bond network without destroying any bonds. While this results in a negligible enthalpy of hydration, the solute constrains some degrees of freedom of neighboring water molecules which gives rise to negative hydration entropy and hence to positive hydration free energy. However, such a simple mechanism has recently come under scrutiny<sup>13,14</sup> because there are simulations<sup>20,21</sup> and theory<sup>22</sup> suggesting that, under some conditions, the hydration of small hydrophobic molecules could be entropically favorable.

The hydration of large hydrophobic particles is believed to occur via a different mechanism.<sup>13,14,23,24</sup> When inserted into liquid water, a large hydrophobe breaks some hydrogen bonds in its vicinity. This would result in large positive hydration enthalpy and hence in a free energy change proportional to

the solute surface area (as opposed to being proportional to the solute volume for small hydrophobes). Thus, the hydration of large hydrophobic particles is expected to be enthalpically driven (and so is their solvent-mediated interaction).

As the thermodynamics of hydration is expected to change gradually from entropic for small solutes to enthalpic for large solutes, so are the structural properties of liquid water in the vicinity of the solutes.<sup>13,14,18,25–29</sup> The small-to-large crossover lengthscale is expected to depend on thermodynamic conditions of the solvent (as well as on the nature of the hydrophobe), such as temperature, pressure, presence of additives, etc... Its dependence on external pressure and concentration of additives was investigated in ref.28 where it was shown to be nanoscopic under ambient conditions but to decrease to molecular sizes upon applying hydrostatic tension or adding ethanol to the solvent (water). On the other hand, the temperature effects on the crossover lengthscale have remained little explored so far. In the present manuscript we will attempt to clarify this particular aspect of multifaceted hydrophobic phenomena.

In order to shed some light on the temperature dependence of the crossover lengthscale of hydrophobic hydration, we will use our previously developed probabilistic hydrogen bond (PHB) approach<sup>30</sup> to water hydrogen bonding that allows one to obtain an analytic expression for the number of bonds per water molecule as a function of both its distance to a hydrophobe and hydrophobe radius. Incorporating that approach into the density functional theory (DFT), we will examine the particle size effects on the hydration of particles over the entire small-to-large lengthscale range at solvent (water) different temperatures. For a hydrophobe of a given size, knowing the temperature dependence of its hydration free energy allows one to calculate the enthalpic and entropic contributions thereto as functions of temperature. Thus, one can obtain the free energy of hydration and its enthalpic and entropic contributions as functions of both temperature and hydrophobe size. The analysis of these function can provide some interesting insight into the temperature dependence of

the crossover lengthscale of hydrophobic hydration.

## 2 Free energy of hydration and its energetic/enthalpic and entropic contributions

The free energy of hydration of a hydrophobic solute of radius  $R$  (the sphericity of the solute being assumed) at temperature  $T$  can be determined as a difference between the values of the appropriate free energy of the system (liquid water) with and without a hydrophobe therein. The free energy of interest must be appropriate for the thermodynamic conditions under which the hydration process takes place.

For example, if hydration occurs at constant temperature  $T$ , volume  $V$ , and number of molecules  $N$  in the solvent (i.e., in a canonical ensemble), the hydration free energy is

$$\Delta F = F - F_0, \tag{1}$$

where  $F$  and  $F_0$  are the Helmholtz free energies of the system (liquid water) with and without a hydrophobe therein, respectively. Likewise, if hydration occurs at constant temperature, volume, and chemical potential  $\mu$  of the solvent (i.e., in a grand canonical ensemble), the hydration free energy is

$$\Delta \Omega = \Omega - \Omega_0, \tag{2}$$

where  $\Omega$  and  $\Omega_0$  are the grand thermodynamic potentials of the system with and without a hydrophobic particle therein, respectively.

It should be noted that in the thermodynamic limit (of  $N \rightarrow \infty$ ,  $V \rightarrow \infty$ ,  $N/V = \text{const}$ ) the hydration free energy can be expected to be independent of whether hydration is canonical or grand canonical or another kind. Indeed, according to the thermodynamic theorem about small perturbations,<sup>31</sup>

the corrections to the internal energy  $E$ , Helmholtz free energy  $F$ , grand thermodynamic potential  $\Omega$ , enthalpy  $W$ , and Gibbs free energy  $G$  are equal to each other if they are calculated at constant variables indicated by the corresponding subscripts in

$$(dE)_{S,V,N} = (dF)_{T,V,N} = (d\Omega)_{T,V,\mu} = (dW)_{S,P,N} = (dG)_{T,P,N} = \sum_i \Lambda_i d\lambda_i, \quad (3)$$

where  $S$  and  $P$  denote the entropy and external pressure of the system, whereas  $dE, dF, d\Omega, dW$ , and  $dG$  are small changes in the thermodynamic potentials occurring in a quasistatic process due to external perturbations. The latter are represented in eq.(4) by the rightmost part,  $\sum_i \Lambda_i d\lambda_i$ , where  $\Lambda_i$  is an intensive (field) variable conjugate to the external constraint  $\lambda_i$  imposed on the system; e.g., such a constraint and its conjugate field variable are responsible for a forced accommodation of a hydrophobic solute in water upon its hydration. We will use this theorem in calculating the temperature dependence of the hydration free energy (see Section 4 below).

Knowing the free energy of hydrophobic hydration, one can find  $\Phi_S$  and  $\Phi_E$ , the entropic and energetic contributions to  $\Delta F$ , as

$$\Phi_S \equiv -T\Delta S = T(\partial\Delta F/\partial T)_{V,N}, \quad \Phi_E \equiv \Delta E = (\partial(\Delta F/T)/\partial(1/T))_{V,N}, \quad (4)$$

respectively, such that  $\Delta F = \Phi_E + \Phi_S$  (in eq.(18) the subscripts of the partial derivatives indicate the thermodynamic variables held constant upon taking the derivatives). Clearly, for the decomposition of  $\Delta F$  into energetic and entropic components it is necessary to know its temperature dependence. This dependence is quite complicated (if not impossible) to obtain by pure analytical means, but one can (numerically or simulationally) calculate the hydration free energy by applying either eq.(1) or eq.(2) for a series of  $T$ 's and  $R$ 's and then construct the functions  $\Delta F = \Delta F(R, T)$  and  $\Delta\Omega = \Delta\Omega(R, T)$  by using interpolation.

### 3 Implementation of the probabilistic hydrogen bond model in the density functional theory

The free energy of hydration of a hydrophobic particle of radius  $R$  in water at temperature  $T$  can be determined by using either computer simulations or DFT.<sup>32–34</sup> As an illustration of the probabilistic hydrogen bond model, let us outline its implementation into DFT which allows one to *explicitly* take into account the effect of water-water hydrogen bonding on the hydrophobe-fluid interactions.

#### 3.1 Weighted density approximation of the density functional theory

Assume that hydration takes place in an open system of constant  $\mu$ ,  $V$ , and  $T$  (grand canonical ensemble). The hydration free energy is then found by using eq.(2). In DFT, the grand thermodynamic potential  $\Omega$  of a nonuniform single component fluid, subjected to an external potential  $U_{\text{ext}}$  (representing a spherical hydrophobe of radius  $R$ ), is a functional of the number density  $\rho(\mathbf{r})$  of fluid molecules

$$\begin{aligned}\Omega[\rho(\mathbf{r})] &= \mathcal{F}_{\text{h}}[\rho(\mathbf{r})] + \frac{1}{2} \int \int d\mathbf{r} d\mathbf{r}' \rho(\mathbf{r}) \rho(\mathbf{r}') \phi_{\text{at}}(|\mathbf{r} - \mathbf{r}'|) \\ &+ \int d\mathbf{r} U_{\text{ext}}(R, \mathbf{r}) \rho(\mathbf{r}) - \mu \int d\mathbf{r} \rho(\mathbf{r}),\end{aligned}\tag{5}$$

where  $\mathcal{F}_{\text{h}}[\rho(\mathbf{r})]$  is the intrinsic Helmholtz free energy functional of hard sphere fluid,  $\mu$  is the chemical potential, and  $\phi_{\text{at}}(|\mathbf{r} - \mathbf{r}'|)$  is the attractive part of the interaction potential between two fluid molecules located at  $\mathbf{r}$  and  $\mathbf{r}'$ ;  $U_{\text{ext}}(R, \mathbf{r})$  is a the external potential whereto a fluid molecule is subjected near the hydrophobe; the integrals are taken over the volume  $V$  of the system. Among various models for  $\mathcal{F}_{\text{h}}[\rho(\mathbf{r})]$ , the weighted density approximation (WDA)<sup>32,35–37</sup> with a weight function independent of weighted density represents an optimal combination of accuracy and simplicity. It is non-local with respect to  $\rho(\mathbf{r})$ ; it takes into account short-ranged correlations and captures the fluid density oscillations near a hard wall.

The key element of WDA is the weighted density  $\tilde{\rho}(\mathbf{r})$  determined as a functional of  $\rho(\mathbf{r})$  via an implicit equation.<sup>32,35,36</sup> The equilibrium density profile is obtained by minimizing  $\Omega[\rho(\mathbf{r})]$  with respect to  $\rho(\mathbf{r})$ . The corresponding Euler-Lagrange equation can be written as

$$\mu = k_B T \ln(\Lambda^3 \rho(\mathbf{r})) + W(\mathbf{r}; \rho(\mathbf{r})), \quad (6)$$

where  $\Lambda = (h^2/2\pi m k_B T)^{1/2}$  is the thermal de Broglie wavelength of a molecule of mass  $m$  ( $h$  and  $k_B$  being Planck's and Boltzmann's constants) and  $W(\mathbf{r}; \rho(\mathbf{r}))$  is a function of  $\mathbf{r}$  and a functional of  $\rho(\mathbf{r})$  (for more details see ref.37).

The hydrophobe being spherical, the external potential is a function of a single variable  $x = r - R$ , and the equilibrium density profile obtained from eq.(6) is a function of a single variable  $r$ :  $\rho(\mathbf{r}) = \rho(r)$ . The substitution of  $\rho(r)$  into eq.(5) provides the grand thermodynamic potential  $\Omega$  of the non-uniform fluid with a hydrophobe therein. The grand thermodynamic potential of uniform liquid water  $\Omega_0$  without a hydrophobe therein can be found by setting  $\rho(\mathbf{r}) = \rho_0$ , where  $\rho_0$  is the equilibrium density of a uniform liquid at given thermodynamic conditions.

### 3.2 Hydrogen bond contribution to the external potential $U_{\text{ext}}$

To apply either DFT or computer simulations to the thermodynamics of hydrophobic phenomena, it is necessary to know the external potential field  $U_{\text{ext}} \equiv U_{\text{ext}}(R, x)$ . Usually, the interaction of fluid molecules with a foreign (impenetrable) substrate is treated in the mean-field approximation whereby every fluid molecule is considered to be subjected to an external potential, due to its pairwise interactions with the substrate molecules.<sup>33,34</sup> The substrate effect on the ability of fluid (water) molecules to form hydrogen bonds had been previously neglected. However, using the PHB model, one can explicitly implement that effect in the DFT formalism.



Indeed, the total external potential can be written as

$$U_{\text{ext}} = U_{\text{ext}}^{\text{p}} + U_{\text{ext}}^{\text{h}}, \quad (7)$$

where  $U_{\text{ext}}^{\text{p}} \equiv U_{\text{ext}}^{\text{p}}(R, x)$  represents the (conventional) *pairwise* potential exerted by all the molecules, constituting the hydrophobe, on a water molecule, and  $U_{\text{ext}}^{\text{h}} \equiv U_{\text{ext}}^{\text{h}}(R, x)$  is the water-water hydrogen bond contribution to  $U_{\text{ext}}$ . This contribution depends on the energy of a single hydrogen bond and number of bonds that a water molecule can form in the hydrophobe vicinity and in the bulk.<sup>37,38</sup> It can be determined as

$$U_{\text{ext}}^{\text{h}} = \frac{1}{2}(\varepsilon_s n_s - \varepsilon_b n_b). \quad (8)$$

$n_b$  and  $\varepsilon_b$  are the number of hydrogen bonds per water molecule and the energy of a bond in the bulk, whereas  $n_s$  and  $\varepsilon_s$  are the analogous quantities in the hydrophobe vicinity. Although  $U_{\text{ext}}^{\text{h}}$  is due to the deviation of  $n_s$  from  $n_b$  as well as the deviation of  $\varepsilon_s$  from  $\varepsilon_b$ ; as previously,<sup>37</sup> the latter effect is neglected hereafter due to its uncertainty.

The first term on the RHS of eq.(8) represents the total energy of hydrogen bonds of a water molecule at a distance  $x$  from the surface of a particle of radius  $R$ , whereas the second term is the energy of its hydrogen bonds in bulk (at  $x \rightarrow \infty$ ); the factor 1/2 is needed to prevent double counting the energy because every hydrogen bond and its energy, either  $\varepsilon_s$  or  $\varepsilon_b$ , are shared between two molecules. Note that the  $R$  and  $x$  dependence of  $U_{\text{ext}}^{\text{h}}$  is determined by the  $R$  and  $x$  dependence of  $n_s$ , while the temperature dependence of  $U_{\text{ext}}^{\text{h}}$  is determined by the temperature dependence of  $n_b$  and  $\varepsilon_b$  (see section 4).

### 3.3 Number of hydrogen bonds per water molecule near a spherical hydrophobic surface

Consider a spherical hydrophobic particle of radius  $R$  immersed in liquid water. Even if one assumes that the intrinsic hydrogen bonding ability of a water molecule is not affected by the hydrophobe, in its vicinity a “boundary” water molecule forms a smaller number of bonds than in bulk because the surface restricts the configurational space available to other water molecules necessary for a boundary water molecule to form hydrogen bonds. The probabilistic model allows one to obtain an analytic expression for the average number of bonds that a BWM can form as a function of its distance to the hydrophobe and hydrophobe radius.

In the probabilistic hydrogen bond model,<sup>39</sup> a water molecule is considered to have four arms each capable of forming a single hydrogen bond. The configuration of four hydrogen-bonding (hb) arms is rigid and symmetric (tetrahedral) with the inter-arm angles  $\alpha = 109.47^\circ$ . Each hb-arm can adopt a continuum of orientations subject to the constraint of tetrahedral rigidity. A water molecule can form a hydrogen bond with another molecule only when the tip of any of its hb-arms coincides with the center of the second molecule. The length of a hb-arm thus equals the length of a hydrogen bond  $\eta$ , assumed independent of whether the molecules are in bulk or near a hydrophobe. The characteristic length of pairwise interactions between water molecules and molecules constituting the hydrophobe is also assumed to be  $\eta$ .

The location of a water molecule is determined by the distance  $r$  from its center to the center of the hydrophobe which is also chosen as the origin of the spherical coordinate system. The distance  $x$  between water molecule and hydrophobe is defined as  $x = r - R$ . A spherical layer of thickness  $\eta$  from  $r = R + \eta$  to  $r = R + 2\eta$  is referred to as the solute hydration layer (SHL).

The number of hydrogen bonds per water molecule near the hydrophobe depends on both  $R$  and  $x$ ,

i.e.,  $n_s = n_s(R, x)$  (the dependence of  $n_s$  on  $T$  is discussed in section 4). the function  $n_s(R, x)$  attains its minimum at  $x = \eta$ , because at this distance the configurational space available for neighboring water molecules is most restricted compared to bulk water. On the other hand, if  $x > 2\eta$ , the number of hydrogen bonds that the water molecule can form is assumed to be unaffected by the hydrophobe:  $n_s(R, x) = n_b$  for  $x \geq 2\eta$ . Thus, according to eq.(8),  $U_{\text{ext}}^h(R, x) \neq 0$  only for  $\eta \leq x \leq 2\eta$ .

In the framework of the PHB approach,<sup>39</sup> the function  $n_s = n_s(R, x)$  is represented as

$$n_s = k_1 b_1 + k_2 b_1^2 + k_3 b_1^3 + k_4 b_1^4, \quad (9)$$

where  $b_1$  is the probability that one of the hb-arms (of a bulk water molecule) can form a hydrogen bond and the coefficients  $k_1, k_2, k_3$ , and  $k_4$  depend on  $R$  and  $x$ , and so does  $n_s$ . Equation (9) assumes that the *intrinsic* hydrogen-bonding ability of a BWM (the tetrahedral configuration of its hb-arms and their lengths and energies) is unaffected by the hydrophobe, but it takes into account the constraint that near the hydrophobe some orientations of the hb-arms of a water molecule cannot lead to the formation of hydrogen bonds. This constraint depends on the distance between water molecule and hydrophobe and on the hydrophobe radius, whence the  $R$ - and  $x$ -dependence of  $k_1, k_2, k_3$ , and  $k_4$ .

The functions  $k_1 \equiv k_1(R, x), k_2 \equiv k_2(R, x), k_3 \equiv k_3(R, x)$ , and  $k_4 \equiv k_4(R, x)$  can be evaluated by using geometric considerations.<sup>30</sup> They all become equal to 1 at  $x \geq 2\eta$ , where eq.(9) reduces to its bulk analog,  $n_b = b_1 + b_1^2 + b_1^3 + b_1^4$  (see ref.39). Since experimental data on  $n_b$  are readily available, one can find  $b_1$  as a positive solution (satisfying  $0 < b_1 < 1$ ) of the latter equation. Thus, equation (9) provides an efficient pathway to  $n_s$  as a function of  $x$  and  $R$  (as well as  $T$ , see the next Section).

## 4 Numerical Calculations

For a numerical illustration, we considered the hydration of spherical hydrophobes of radii  $R/\eta = 1, 3, 5, 7, 10, 15, 20, 30, 50, 100$  in the model water at five temperatures,  $T = 293.15$  K,  $303.15$  K,  $313.15$  K,  $323.15$  K, and  $333.15$  K, the chemical potential being the same at all temperatures,  $\mu = -11.5989 k_B T_0$  (with  $T_0 = 293.15$  K). Hydration was assumed to occur at constant  $\mu, V, T$ , so that the hydration free energy was determined as the change in the grand canonical potential of the system,  $\Delta\Omega$ , by using the combined PHB/DFT formalism as outlined above. According to the thermodynamic theorem about small corrections, eq.(3), one can then set  $\Delta F \approx \Delta\Omega$  and carry out the decomposition of the hydration free energy into its enthalpic and entropic contributions by using eq.(4).

As clear from the foregoing, the temperature dependence of  $\Delta\Omega$  (or  $\Delta F$ ) contains a contribution from the temperature dependence of  $U_{\text{ext}}^{\text{h}}$ , hydrogen bond contribution to the total external field. The dependence of  $U_{\text{ext}}^{\text{h}}$  on  $T$  is due to the temperature dependence of four quantities:  $n_s$ ,  $n_b$ ,  $\epsilon_s$ , and  $\epsilon_b$  (see eq.(8)). The functions  $\epsilon_b \equiv \epsilon_b(T)$  and  $n_b \equiv n_b(T)$  are either readily available or can be constructed on the basis of available data. Therefore, by virtue of eq.(9), the dependence of  $n_s$  on  $T$  can be considered to be known as well. The energy of a hydrogen bond was assumed to depend on temperature in such a way that  $\epsilon_s(T)/\epsilon_b(T) \approx \text{const}$  in the temperature range considered. One can thus consider  $U_{\text{ext}}^{\text{h}}$  to be a known function of not only  $x$  and  $R$ , but also  $T$ :  $U_{\text{ext}}^{\text{h}} = U_{\text{ext}}^{\text{h}}(R, x, T)$ . This allows one to numerically determine the temperature dependence of the free energy of hydration and to subsequently use interpolation procedure to find an analytical fit thereof which then can be used in eq.(4).

The liquid state of bulk water was ensured by imposing the appropriate boundary condition onto eq.(6),  $\rho(x) \rightarrow \rho_l$  as  $x \rightarrow \infty$ , with  $\rho_l$  the bulk liquid density. The densities  $\rho_v$  and  $\rho_l$  of coexisting vapor and liquid, respectively, are determined by solving the equations  $\mu(\rho, T)|_{\rho=\rho_v} =$

$\mu(\rho, T)|_{\rho=\rho_l}$ ,  $p(\rho, T)|_{\rho=\rho_v} = p(\rho, T)|_{\rho=\rho_l}$ , requiring the chemical potential  $\mu \equiv \mu(\rho, T)$  and pressure  $p \equiv p(\rho, T)$  to be the same throughout both coexisting phases. The liquid densities for the above five temperatures thus obtained were  $\rho\eta^3 = 0.6342, 0.6517, 0.6647, 0.6750$ , and  $0.6835$ , respectively.

Solving eq.(6), the chemical potential of a uniform hard sphere fluid  $\mu_h$  and the configurational part  $\Delta\psi_h \equiv \Delta\psi_h(\rho, T)$  of the free energy of a hard sphere fluid were modeled in the Carnahan-Starling approximation,<sup>33,34,40</sup> whereas for the weight function  $w(|\mathbf{r}' - \mathbf{r}|; \tilde{\rho}(\mathbf{r}))$  (entering in the implicit equation that determines  $\tilde{\rho}(\mathbf{r})$  as a functional of  $\rho(\mathbf{r})$ ) we adopted a  $\tilde{\rho}$ -independent version,<sup>36</sup>

$$\mu_h = k_B T \left( \ln(\Lambda^3 \rho) + \xi \frac{8 - 9\xi + 3\xi^2}{(1 - \xi)^3} \right), \quad \Delta\psi_h = k_B T \frac{\xi(4 - 3\xi)}{(1 - \xi)^2}, \quad w(r_{12}) = \frac{3}{\pi\eta^4}(\eta - r_{12})\Theta(\eta - r_{12}),$$

with  $\xi = (\pi d^3/6)\rho$  and  $\Theta(u)$  being the Heaviside (unit-step) function (the quantities  $\mu_h$ ,  $\Delta\psi_h$ , and  $w(|\mathbf{r}' - \mathbf{r}|; \tilde{\rho}(\mathbf{r}))$  are all needed<sup>30,35–37</sup> to calculate  $W(\mathbf{r}; \rho(\mathbf{r}))$  in eq.(6)).

The pairwise interactions of water molecules were modeled by using the Lennard-Jones (LJ) potential with the energy parameter  $\varepsilon_{ww} = 3.79 \times 10^{-14}$  erg and the diameter  $d$  of a model molecule set to be  $\eta$ . The attractive part  $\phi_{at}$  of pairwise water-water interactions was modeled via the Weeks-Chandler-Anderson perturbation scheme.<sup>41</sup> The interaction potential between water molecule and molecule of a hydrophobe was assumed to be of LJ type with an energy parameter  $\varepsilon_{wp}$  and a length parameter  $\eta$ . Integrating this interaction with respect to the position of the molecule of the hydrophobe over the hydrophobe volume  $V_R = 4\pi R^3/3$ , one can obtain the pairwise contribution  $U_{ext}^p$  into  $U_{ext}$ . The dimensionless number density of molecules in the hydrophobe was set to be  $\rho_p\eta^3 \approx 1$ . The density profiles and hydration free energies and its enthalpic and entropic component thus obtained are shown in Figures 1 through 5.

Figure 1 presents the density profiles near a spherical hydrophobe of radius  $R$  with the degree of hydrophobicity  $\varepsilon_{wp}/\varepsilon_{ww} = 0.75$ . Different figure panels show results for different radii

( $R/\eta = 1, 3, 5, 7, 10, 15, 30, 100$ ). In each panel, different curves correspond to different temperatures ( $T = 293.15, 303.15, 313.15, 323.15, 333.15$  from bottom to top, respectively). As clear, both the temperature and the hydrophobe radius greatly affect the distribution of vicinal water molecules.

At lower temperatures  $T = 293.15$  K, and 303.15 K, the oscillations in the density profile gradually disappear as  $R$  increases. They are well pronounced for  $R/\eta = 1$ , but virtually non-existent for particles  $R/\eta \geq 7$ . As  $R$  increases, a thin depletion layer around the particle (virtually non-existent for  $R/\eta = 1$ ) becomes more developed, with its density approaching that of vapor and its thickness approaching  $\eta$ . However, at higher temperatures, the density oscillations are present in the vicinity of hydrophobes of all sizes. On the other hand, the higher the temperature the narrower the thickness of the depletion layer near a hydrophobe and the higher the fluid density therein. These latter effects have a clear lengthscale dependence.

Indeed, for a hydrophobe of  $R/\eta = 1$  the temperature increase from  $T = 293.15$  to  $T \gtrsim 303.15$  leads to the increase of the “contact” fluid density (i.e., the fluid density at contact with the hydrophobe) by many orders of magnitude, i.e., the vapor-like depletion layer transforms into a liquid like depletion layer whereof the thickness also quickly decreases with increasing temperature. At  $T = 333.15$  K the contact fluid density equals about  $0.5\rho_l$  and the width of the depletion layer equals about  $0.3\eta$ ; the corresponding quantities at  $T = 293.15$  K are equal to about  $0.01\rho_l$  and  $0.7\eta$ . On the other hand, for a hydrophobe of  $R/\eta = 3$ , at  $T = 333.15$  K the contact fluid density equals about  $0.02\rho_l$  and the width of the depletion layer equals about  $0.67\eta$ , whereas at 293.15 the corresponding quantities are about  $0.0003\rho_l$  and  $0.88\eta$ . Thus, the temperature dependence of these two characteristics of the fluid density profile near a hydrophobe is very sensitive to the hydrophobe size. This sensitivity is consistent with the largely accepted view that the underlying physics of hydrophobicity is different on different length scales.

Figure 2a, 2b, and 2c present the free energy of hydrophobic hydration and its entropic and en-

thalpic constituents, respectively, vs temperature (note that the curves are provided only for guiding the eye; the actual calculated points are at  $T = 293.15, 303.15, 313.15, 323.15, 333.15$ ). The curves correspond to  $R/\eta = 1, 3, 5, 7, 10, 15, 20, 30, 50$ , and 100 (from bottom to top in Figs.2a and 2c, from top to bottom in Fig.2b). The intrinsic hydrophobicity of the particles is assumed to be independent of  $R$ , with  $\varepsilon_{\text{wp}}/\varepsilon_{\text{ww}} = 0.75$ . The hydration free energy and its constituents are expressed in units of  $k_B T_1$  per “dimensionless unit area”; the dimensionless quantities  $\overline{\Delta\Omega}$ ,  $\overline{\Phi_S}$ , and  $\overline{\Phi_E}$  are defined as  $\overline{\Delta\Omega} = \Delta\Omega/(k_B T_1(4\pi R^2/\eta^2))$ ,  $\overline{\Phi_S} = \Phi_S/(k_B T_1(4\pi R^2/\eta^2))$ , and  $\overline{\Phi_E} = \Phi_E/(k_B T_1(4\pi R^2/\eta^2))$ , respectively.

As clear from this Figure, in the considered temperature range from 293.15 K to 333.15 K both the hydration free energy and its entropic constituent increase with temperature for a hydrophobe of any size. One can note that the sensitivity of both  $\overline{\Delta\Omega}$  and  $\overline{\Phi_S}$  increases with decreasing  $R$ . In this temperature range, one can use a linear fit in constructing an analytic expression on the basis of numerical results for the temperature dependence of the hydration free energy (at a given  $R$ ). Consequently, the entropic constituent  $\overline{\Phi_S}$  thereof will be linear dependent on  $T$ , while its enthalpic component  $\overline{\Phi_E}$  will be roughly independent of  $T$ . A more accurate fitting function for the data  $\Delta\Omega$  vs  $T$  (at given  $R$ ) would result in a  $T$ -dependent enthalpic component  $\Phi_E$ . However, the results in Fig.2c suggest that  $\Phi_E$  can be expected to be a relatively weak function of  $T$  in the whole small-to-large range of hydrophobe length scales.

Figures 3a, 3b, and 3c present the free energy of hydrophobic hydration  $\Delta F$  and its enthalpic and entropic constituents, respectively, as functions of the hydrophobe radius  $R$  (note that the curves are provided only for guiding the eye; the actual calculated points are at  $R/\eta = 1, 3, 5, 7, 10, 15, 20, 30, 50$ , and 100). The intrinsic hydrophobicity of the particles is assumed to be independent of  $R$ , with  $\varepsilon_{\text{wp}}/\varepsilon_{\text{ww}} = 0.75$ . The hydration free energy and its constituents are shown as the dimensionless quantities  $\overline{\Delta\Omega}$ ,  $\overline{\Phi_S}$ , and  $\overline{\Phi_E}$ . Five different curves in Figs.3a and 3b correspond to five different

temperatures,  $T = 293.15, 303.15, 313.15, 323.15, 333.15$  (from bottom to top). The five curves merge into one in Fig.3c as the enthalpic contribution  $\overline{\Phi_E}$  is independent of  $T$  due to a linear approximation used for the  $T$  dependence of the hydration free energy.

The variable sensitivity of  $\overline{\Delta\Omega}$ ,  $\overline{\Phi_S}$ , and  $\overline{\Phi_E}$  to  $R$  is another indication that the hydration of small and large length-scale particles occur via different mechanisms. For small hydrophobes, they increase sharply with increasing  $R$ , but become weakly sensitive to  $R$  for large enough hydrophobes. As expected, the always-positive hydration free energy  $\overline{\Delta\Omega}$  increases with increasing  $R$ , quite sharply for small hydrophobes (with  $R/\eta \lesssim 7$ ), but relatively weakly for large ones (with  $R/\eta \gtrsim 10$ ); its always-positive entropic contribution  $\overline{\Phi_S}$  behaves in the inverse (decreasing with increasing  $R$ ) manner on the same length scales. The enthalpic contribution  $\overline{\Phi_E}$  is an increasing function of  $R$ . For small hydrophobes it is negative but sharply increases with increasing  $R$ , becoming zero at some  $R_E^\pm$  (in our calculation we could estimate  $R_E^\pm/\eta$  to be about 6.34) and positive for  $R > R_E^\pm$ . Note that in the region of negative  $\overline{\Phi_E}$ , i.e. for  $0 < R \lesssim R_E^\pm$ , both contribution are close by absolute value with the entropic contribution just slightly dominant, which leads to relatively small value of the hydration free energy even there where its components  $\overline{\Phi_E}$  and  $\overline{\Phi_S}$  have large (negative and positive, respectively) values. This represents a well-established characteristic feature of the hydration of molecular scale apolar solutes.

Furthermore, there exists such a radius  $R_{SE}$  that  $\overline{\Phi_E} \gtrless \overline{\Phi_S}$  for  $R \gtrless R_{SE}$ . This radius is sometimes referred to as a crossover lengthscale of hydrophobic hydration. For hydrophobes of radii smaller than  $R_{SE}$  the hydration free energy is dominated by its entropic component ( $\overline{\Phi_E} < \overline{\Phi_S}$ ), whereas for hydrophobes of radii larger than  $R_{SE}$  the hydration free energy is dominated by its enthalpic constituent ( $\overline{\Phi_E} > \overline{\Phi_S}$ ). The crossover radius can be determined as a solution of the equation  $\overline{\Phi_E}(T, R) = \overline{\Phi_S}(T, R)$ . Clearly, it is a function of temperature, i.e.,  $R_{SE} = R_{SE}(T)$ . Figure 4 shows this function for the hydration of solutes of given hydrophobicity  $\varepsilon_{wp}/\varepsilon_{ww} = 0.75$  in the



temperature range from 293.15 K to 333.15 K. Note that this  $T$ -dependence of  $R_{SE}$  results from the linear approximation of the  $T$  dependence of the hydration free energy; more accurate approximation for the latter will certainly affect the function  $R_{SE} = R_{SE}(T)$  as well.

The temperature dependence of the hydration free energy predicted by the combined PHB/DFT model for small scale hydrophobes is in a reasonable agreement with both experimental data and simulational results reported for simple solutes.<sup>42–44</sup> Figure 5 presents such a comparison for the temperature dependence of the hydration free energy. The three short solid lines correspond to the predictions of the PHB/DFT model for solutes of radii  $R/\eta = 1, 3, 5$  (from bottom to top, respectively) and of hydrophobicity  $\varepsilon_{wp}/\varepsilon_{ww} = 0.75$ . The filled circles and squares represent the experimental data<sup>42</sup> for the molecules of xenon and methane, respectively. The results obtained<sup>43</sup> by molecular dynamics simulations for the hydration of neon, argon, krypton, xenon, and methane molecules in an SPCE model water are shown as short-dashed, long-dashed, short-long-dashed, dash-dotted, and dotted curves, respectively. Converting the original data from refs.42 and 43 to the dimensionless hydration free energy per dimensionless unit area, we set the radii of the solute molecules to their corresponding van der Waals radii as provided on the web site [www.wikipedia.org](http://www.wikipedia.org); the ratio  $R/\eta$  thus was 0.46 for neon, 0.59 for argon, 0.637 for krypton, 0.68 for xenon, and 0.644 for methane. Note also that the discrepancies between the simulated data and the PHB/DFT predictions can be partially accounted for by the differences in the ratio  $\varepsilon_{wp}/\varepsilon_{ww} = 0.75$  used in the latter and the corresponding ratios used in the simulations (0.237 for neon, 1.598 for argon, 2.16 for krypton, 2.75 for xenon, and 1.89 for methane). Taking into account these caveats as well as the discrepancies between the results obtained via MD simulations for different water models<sup>43</sup> (SPC, SPCE, TIP3P, TIP4P, TIP5P), one can deem the predictions of the PHB/DFT model in qualitatively satisfactory agreement with both experimental and simulational analogues.

## 5 Conclusions

The thermodynamics of hydration is expected to change gradually from entropic for small hydrophobic solutes to enthalpic for large ones. The range of solute linear sizes over which the crossover of the hydration regime is expected to occur depends not only on the nature of the hydrophobe but also on the thermodynamic conditions of the solvent such as temperature, pressure, presence of additives, etc...

In order to elucidate some molecular and thermodynamic aspects of the crossover properties of hydrophobic hydration, including its crossover lengthscale, we have used the combination of our previously developed probabilistic hydrogen bond model with the density functional theory. The probabilistic approach to water hydrogen bonding allows one to obtain an analytic expression for the number of bonds per water molecule as a function of both its distance to a solute and solute radius. This function allows one to explicitly incorporate the effect of water-water hydrogen bonding, crucial element of hydrophobic phenomena, on the water-hydrophobe interactions into the density functional theory. One can thus examine the solute size effects on its hydration over the entire small-to-large lengthscale range at a series of different temperatures. For a hydrophobe of given size, knowing the temperature dependence of its hydration free energy allows one to calculate the enthalpic and entropic contributions thereto as functions of temperature. Thus, one can obtain the free energy of hydration and its enthalpic and entropic contributions as functions of both temperature and hydrophobe size. The analysis of these function can provide some interesting insight into the temperature dependence of the crossover lengthscale of hydrophobic hydration.

As a numerical illustration of the combined PHB/DFT approach, we have studied the hydration of spherical particles of various radii and of fixed hydrophobicity in a model water in the temperature range from 293.15 K to 333.15 K. The predictions for the temperature of the hydration free energy

of small size hydrophobes are qualitatively and even quantitatively consistent with the experimental and simulational data on the hydration of simple molecular solutes (neon, argon, krypton, xenon, and methane). The model predicts that indeed, there exists such a “crossover” radius that for a hydrophobe of this size the enthalpic and entropic contributions to its hydration free energy are equal; for smaller hydrophobes the entropic contribution dominates, whereas for larger hydrophobes the enthalpic one does. The temperature dependence of this crossover radius was found to be accurately enough fit with a quadratic polynomial in  $T$ . However, this may be a consequence of the linear approximation used to construct an analytical function for the temperature dependence of the hydration free energy on the basis of numerical results. More accurate numerical calculations with more intermediate points in the temperature range considered may warrant a different fit for the temperature dependence of the hydration free energy which will also affect the temperature dependence of the crossover radius.

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## Captions

to Figures 1 to 5 of the manuscript “ TEMPERATURE EFFECT ON THE SMALL-TO-LARGE CROSSOVER LENGTHSCALE OF HYDROPHOBIC HYDRATION ” by **Y. S. Djikaev** and **E. Ruckenstein**.

Figure 1. Density profiles near a spherical hydrophobe of radius  $R$  and  $\varepsilon_{wp}/\varepsilon_{ww} = 0.75$ . Different figure panels show results for different radii ( $R/\eta = 1, 3, 5, 7, 10, 15, 30, 100$ ). In each panel, different curves correspond to different temperatures ( $T = 293.15, 303.15, 313.15, 323.15, 333.15$  from bottom to top, respectively). The imposed chemical potential is  $\mu = -11.5989 k_B T_1$  (see the text for more details).

Figure 2. Free energy of hydration and its entropic and enthalpic constituents as functions of temperature, for hydrophobes of different radii with  $\varepsilon_{wp}/\varepsilon_{ww} = 0.75$ . The curves correspond to  $R/\eta = 1, 3, 5, 7, 10, 15, 20, 30, 50$ , and  $100$  (from bottom to top in Figs.2a and 2c, from top to bottom in Fig.2b) The hydration free energy and its entropic and enthalpic components are shown as the dimensionless quantities  $\overline{\Delta\Omega}$ ,  $\overline{\Phi_S}$ , and  $\overline{\Phi_E}$ , respectively (see the text for definitions).

Figure 3. Free energy of hydrophobic hydration  $\Delta F$  and its enthalpic and entropic constituents as functions of the hydrophobe radius  $R$  with  $\varepsilon_{wp}/\varepsilon_{ww} = 0.75$ . The hydration free energy and its constituents are shown as the dimensionless quantities  $\overline{\Delta\Omega}$ ,  $\overline{\Phi_S}$ , and  $\overline{\Phi_E}$ . The curves correspond to five different temperatures,  $T = 293.15, 303.15, 313.15, 323.15, 333.15$  (from bottom to top); they merge into one in Fig.3c as the enthalpic contribution  $\overline{\Phi_E}$  is independent of  $T$  (see the text for details).

Figure 4. Temperature dependence of the small-to-large crossover radius  $R_{SE}$  for the hydration of solutes of given hydrophobicity  $\varepsilon_{wp}/\varepsilon_{ww} = 0.75$ , obtained in the linear approximation for the dependence of  $\Delta\Omega$  on  $T$ .

Figure 5. Comparison of the temperature dependence of the hydration free energy predicted by the combined PHB/DFT model for small scale hydrophobes with both experimental data and simulation results for simple solutes. The three short solid lines are the model predictions for solutes of radii  $R/\eta = 1, 3, 5$  (from bottom to top, respectively) with  $\varepsilon_{wp}/\varepsilon_{ww} = 0.75$ . The filled circles and squares represent the experimental data for xenon and methane, respectively.<sup>42</sup> The results by MD simulations of neon, argon, krypton, xenon, and methane molecules in the SPCE model water are shown as short-dashed, long-dashed, short-long-dashed, dash-dotted, and dotted curves, respectively.<sup>43</sup> The hydration free energy and its entropic and enthalpic components are shown as the dimensionless quantities  $\overline{\Delta\Omega}$ ,  $\overline{\Phi_S}$ , and  $\overline{\Phi_E}$ , respectively (see the text for details)



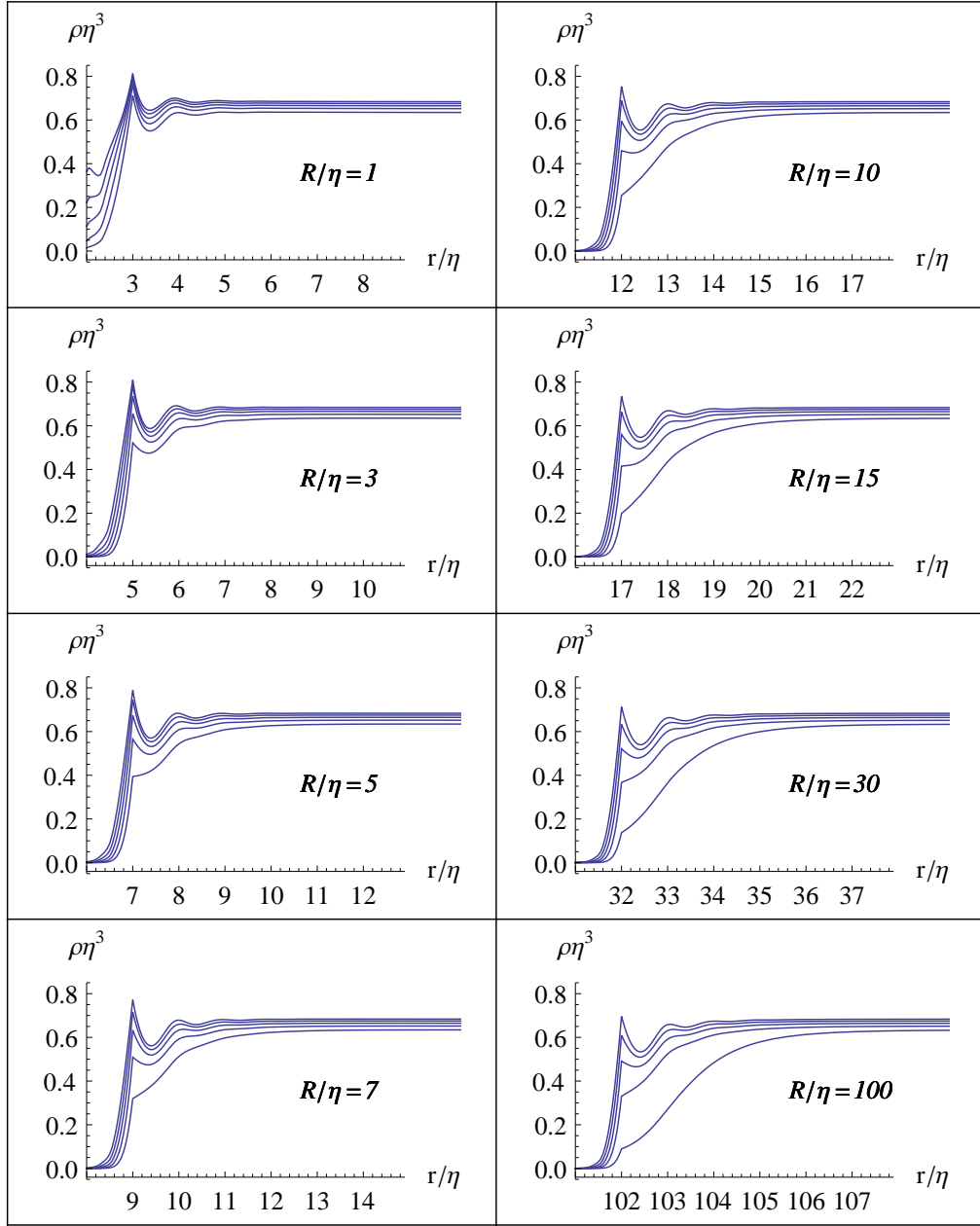


Figure 1:

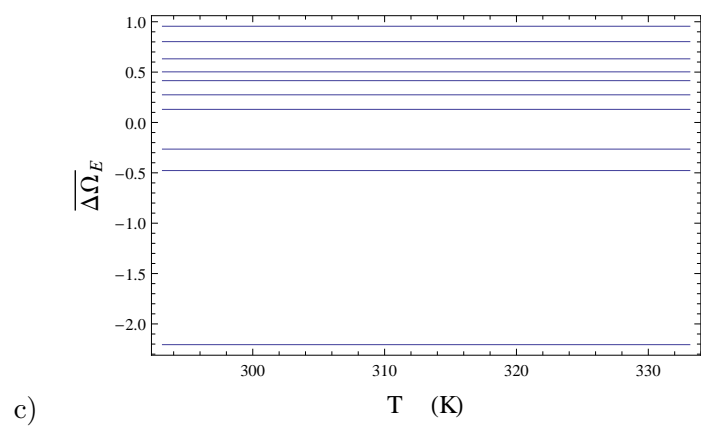
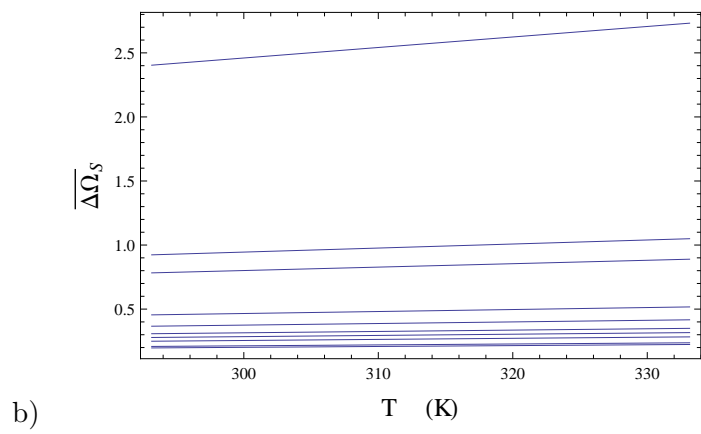
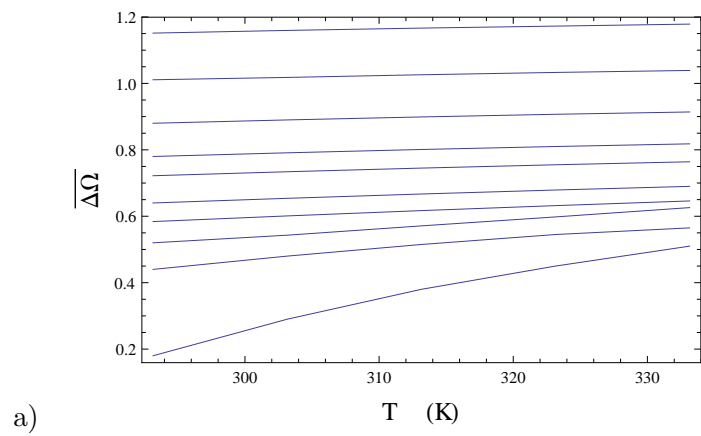


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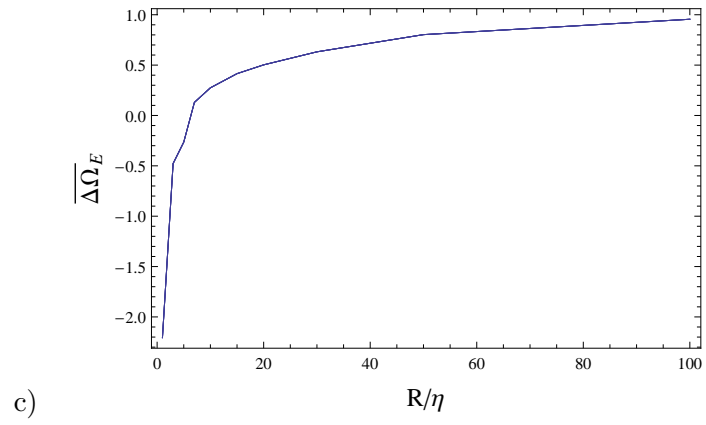
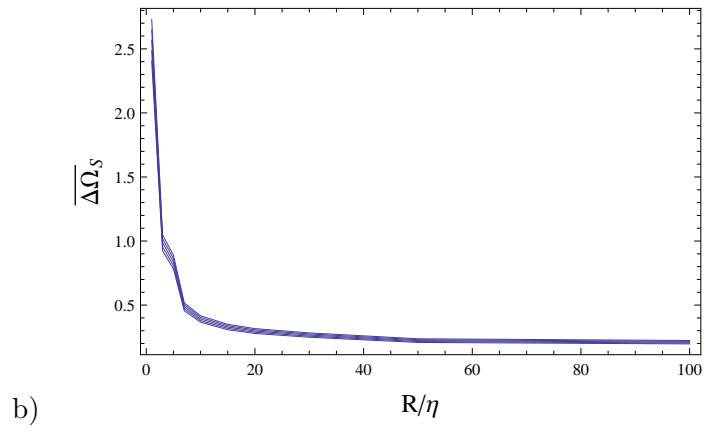
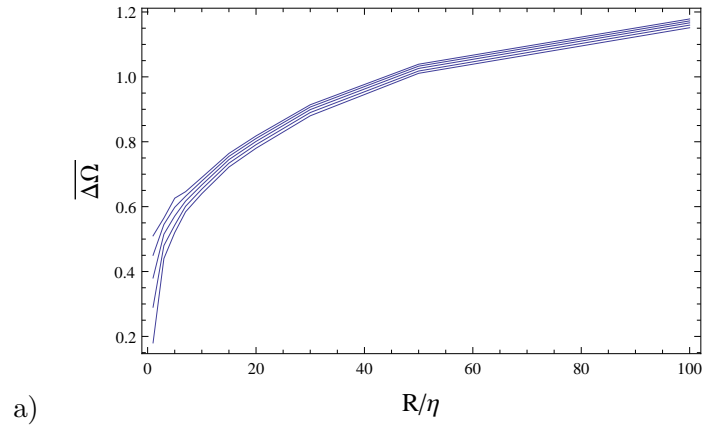


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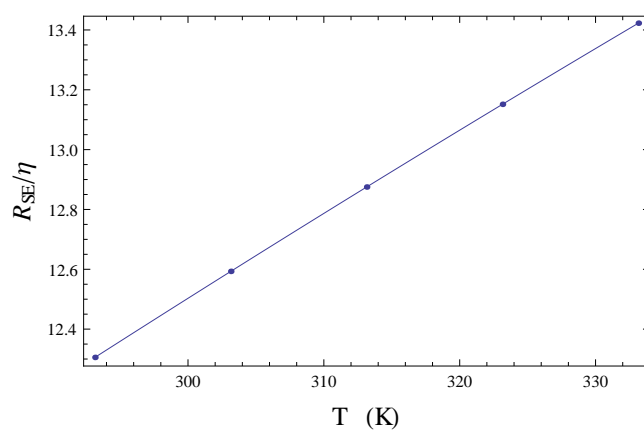


Figure 4:

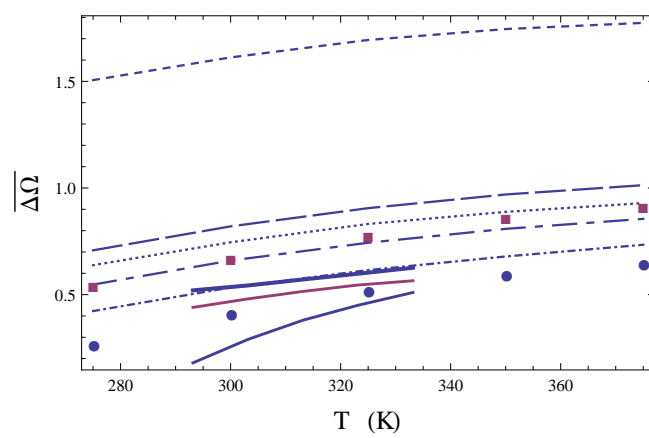


Figure 5: